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Isolation of the first toxin from the scorpion *Buthus occitanus israelis* showing preference for *Shaker* potassium channels

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Abstract **We have purified BoiTx1, the first toxin from the venom of the Israeli scorpion, *Buthus occitanus israelis*, and studied its activity and genomic organization. BoiTx1 is a 37 amino acid-long peptide contained six conserved cysteines, and is classified as an α-KTx3.10 toxin. The pharmacological effects of BoiTx1 were studied on various cloned K^+ channels expressed in *Xenopus laevis* oocytes. BoiTx1 inhibited currents through *Drosophila Shaker* channels with an IC_{50} value of 3.5 ± 0.5 nM, yet had much lesser effect on its mammalian orthologs. Thus, BoiTx1 is the first member of the α-KTx3 family that preferentially affects insect potassium channels.**

Keywords: α-KTx3; Potassium channels; Scorpion toxin; *Shaker*; TEVC; *Buthus occitanus*

1. Introduction

Since the isolation of noxiustoxin, the first short chain potassium channel-blocking toxin from the venom of *Centruroides noxius* [1,2], scorpion venoms have been considered as rich sources of potassium channel-blocking peptides [3–5]. Potassium channel-blocking toxins are typically 23–64 amino acid residues in length and well-packed by either three or four disulfide bridges. Toxin peptides are structurally related, with a structural signature defined by the presence of the cysteine-stabilized α/β motif, in which two disulfide bridges covalently link a α-helical segment with one strand of a β-sheet structure [3–5]. To date, more than 120 potassium channel modifiers have been discovered and are divided into four major families (α-, β-, γ- and κ-KTx's), grouped according to their primary sequence [5]. α-KTx's can, in turn, be divided into more than 20 independent sub-families [5–7].

Characteristic of scorpion toxins is their ability to distinguish between different channel sub-types and even between orthologous channels [8]. Moreover, several sodium channel modifiers were shown to possess impressive selectivity towards various organisms, with the most studied being the anti-insect-selective sodium channel modifiers [9–11].

There are nine known members of the α-KTx3 toxin group, agents that block certain *Shaker* and *Shaker*-like Kv1.*x* voltage-gated potassium channels as well as some calcium-activated potassium channels [12–15]. Here, we describe the isolation and cloning of the first toxin from the Israeli scorpion, *Buthus occitanus israelis* [16]. The toxin, termed BoiTx1, is a new member of the α-KTx3 toxin family and is at least a hundredfold more potent against the fly *Shaker* channel than towards its mammalian homologs, Kv1.1 and Kv1.3.

2. Materials and methods

2.1. Toxin purification

B. occitanus israelis scorpions were collected in the Negev desert of Israel and crude venom was extracted by electrical stimulation of the venom gland [17]. Crude venom from 40 scorpions was lyophilized, suspended in 200 μl of 10 mM ammonium acetate and centrifuged at 13,000 × *g* for 15 min at 4 °C. The pellet was extracted two more times and the combined supernatants were filtered through a 0.2 μm filter.

High performance liquid chromatography (HPLC) analyses were performed on an AKTAbasic purifier (Amersham Biosciences). Crude venom was separated on a 3 ml Resource RPC column (Amersham Biosciences) using buffer A (0.1% trifluoroacetic acid (TFA) in water) and buffer B (0.1% TFA in acetonitrile) in the following gradient: 0–8% buffer B (6 min), 8–45% buffer B (36 min) and finally, 45–100% buffer B (6 min). Fractions were lyophilized and dissolved in 20 mM ammonium acetate (buffer A). Active fractions were applied to a 1 ml Resource S cation exchange column (Amersham Biosciences), using 1 M NaCl in 20 mM ammonium acetate as buffer B. Separation was performed using buffers A and B in a linear gradient of 0–60% buffer B (18 min) followed by a linear gradient of 60–100% buffer B (2 min). Final purification of the active fractions were performed using a Vydac C18 (4.6 × 250 mm) column using buffer A (0.1% TFA in water) and buffer B (0.1% TFA in acetonitrile) in the following gradient: 5–10% buffer B (2 min), 10–40% buffer B (62 min) and finally, 40–100% buffer B (8 min).

2.2. Mass spectrometry analysis and amino acid sequencing

The molecular weights of the purified and chymotrypsin-digested toxin were determined using matrix-assisted laser desorption ionization-time-of-flight (MALDI-TOF) spectroscopy (Reflex IV, Bruker Daltonics), performed at the Biotechnology Institute of Ben-Gurion University. Toxin preparation for digestion included successive denaturation, reduction and alkalization using 8 M urea, 450 mM dithiothreitol (DTT) and 500 mM iodoacetamide at room temperature for 20 min. Chymotryptic digestion of the treated toxin was carried out in digestion buffer containing 10 mM $CaCl_2$, 100 mM Tris–HCl pH 7.8 at 30 °C over night.

The first five amino acid sequence of the toxin was determined via Edman degradation, carried out by the Protein Microsequencing Facility of the Weizmann Institute (Rehovot, Israel).

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Abbreviations: MALDI-TOF, matrix-assisted laser desorption ionization–time-of-flight; HPLC, high performance liquid chromatography; KTx, K^+ channel toxin; Kv, voltage-gated K^+ channel; TFA, trifluoroacetic acid; DTT, dithiothreitol; TEVC, two-electrode voltage clamp technique; BSA, bovine serum albumin

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2.3. Electrophysiology activity assay

Xenopus laevis oocytes were isolated, defolliculated and maintained at 17 °C in ND-91 solution (in mM): 91 NaCl, 2 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, and 5 HEPES, pH 7.5, supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin and 50 μg/ml gentamycin. Oocytes were injected with 23 or 46 nl, containing 0.2–15 ng of cRNA. Whole-cell currents were measured 1–3 days after injection by the two-electrode voltage clamp technique (TEVC), using a GeneClamp 500B amplifier (Axon Instruments). Data were filtered at 0.5 kHz and sampled at 2 kHz with Clampex 9.0 software (Axon Instruments). The pipette was filled with 3 M KCl and the bath solution contained (in mM): 4 KCl, 96 NaCl, 1 $MgCl_2$, 0.3 $CaCl_2$, 5 HEPES, pH 7.4. For activity measurements, the toxin was suspended in 20 mM ammonium acetate supplemented with 100 μg/ml bovine serum albumin (BSA), diluted in the external solution supplemented with 100 μg/ml BSA and added directly to the chamber.

2.4. cDNA library construction and screening

Total RNA was extracted 3 days after electrical 'milking' of the venom glands, using the EZ RNA extraction kit (Promega), while mRNA was purified using the PolyATract mRNA Isolation System (Promega). Double-stranded cDNA was synthesized from 2 μg of mRNA with the Universal Riboclone cDNA Synthesis System (Promega). cDNA was cloned into pBluescript KS^+ plasmids digested with *SmaI* and transfected into *Escherichia coli* DH5α cells. Three hundred randomly chosen cDNAs, ranging in length from 250 to 600 bp, were sequenced to obtain a reliable representation of the toxin content in the venom gland.

2.5. DNA preparation and amplification

Genomic DNA was purified from abdomen segments of three scorpions using an EZ DNA kit (Bet Haemek, Israel). Primers used for PCR amplification were designed to match the 5′- and 3′-untranslated regions of the cDNA. The forward primer was 5′-GGGTTTATTTGTAAAAATATTGCG-3′ and the reverse primer was 5′-AATTAAAAGCAATTTATTGTATTC-3′. Genomic DNA was amplified using Phusion DNA polymerase (Finnzymes). PCR products were phosphorylated, ligated into pBluescript KS^+ and sequenced.

3. Results

3.1. Toxin purification

Crude venom (9 mg) was separated using reverse-phase chromatography into approximately 25 fractions (Fig. 1A). The fraction eluted at 20.5 ml, accounting for 11% of the total absorbance at 220 nm, was the most active against *Shaker* channels and further purified. The active fractions from four reverse-phase separations were pooled and further purified using a Resource S column (Fig. 1B), yielding two main peptides. The fraction eluted at 13.9 ml was further purified on a Vydac analytical C18 column (Fig. 1C). Approximately, 27 μg of pure toxin, termed BoiTx1, was eluted at 24.6 ml.

3.2. Characterization and structural analysis of BoiTx1

Out of 300 analyzed cDNA clones, 150 were predicted to encode for toxin-like peptides (not shown). The expected molecular weights of the predicted toxins were calculated to create a database of expected masses. To determine the amino acid sequence of BoiTx1, the molecular mass of the toxin was defined by MALDI-TOF and found to be 4015.6 Da (Fig. 2A). In addition, the same analysis was applied to the toxin after reduction and digestion with chymotrypsin (Fig. 2A). The

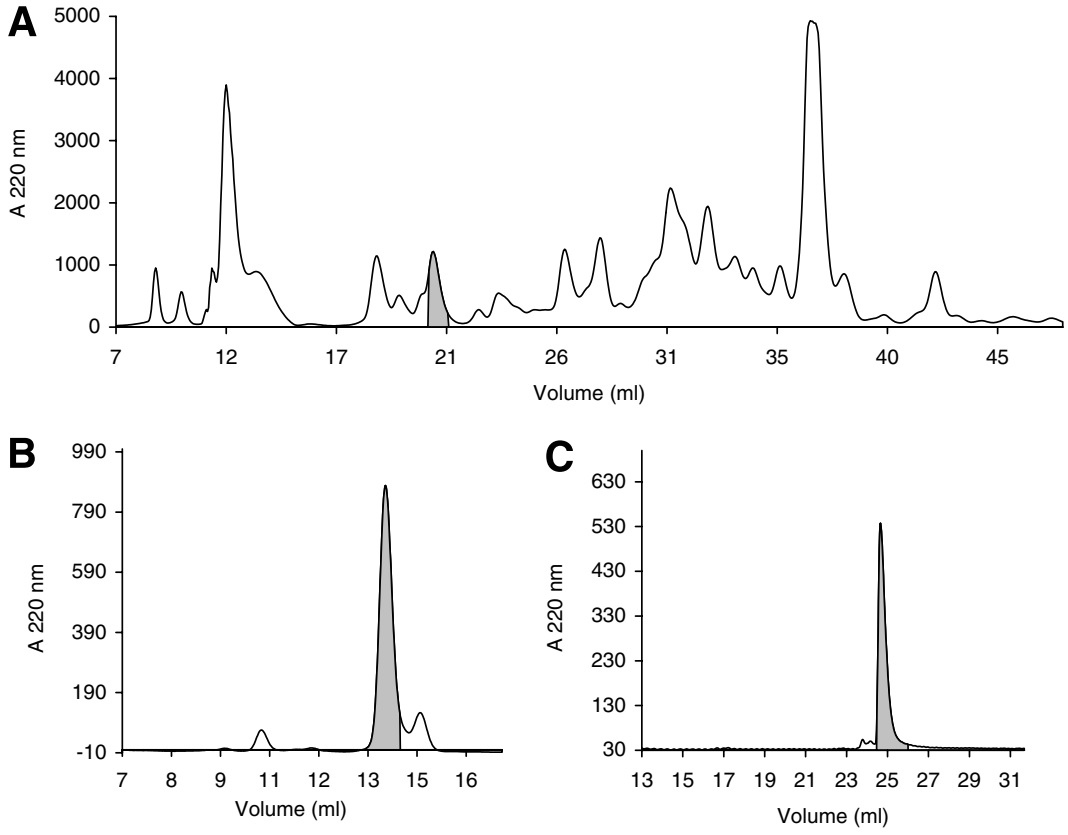

Fig. 1. Purification of BoiTx1 from the venom of *Buthus occitanus israelis*. Venom was dissolved in 0.1% TFA, filtered and BoiTx1 was isolated by three successive steps, using (A) a 3 ml RPC column (Amersham Biosciences), (B) a 1 ml Resource S cation exchange column (Amersham Biosciences) and (C) a Vydac C18 (4.6 × 250 mm) column. BoiTx1-containing fractions are marked in gray.

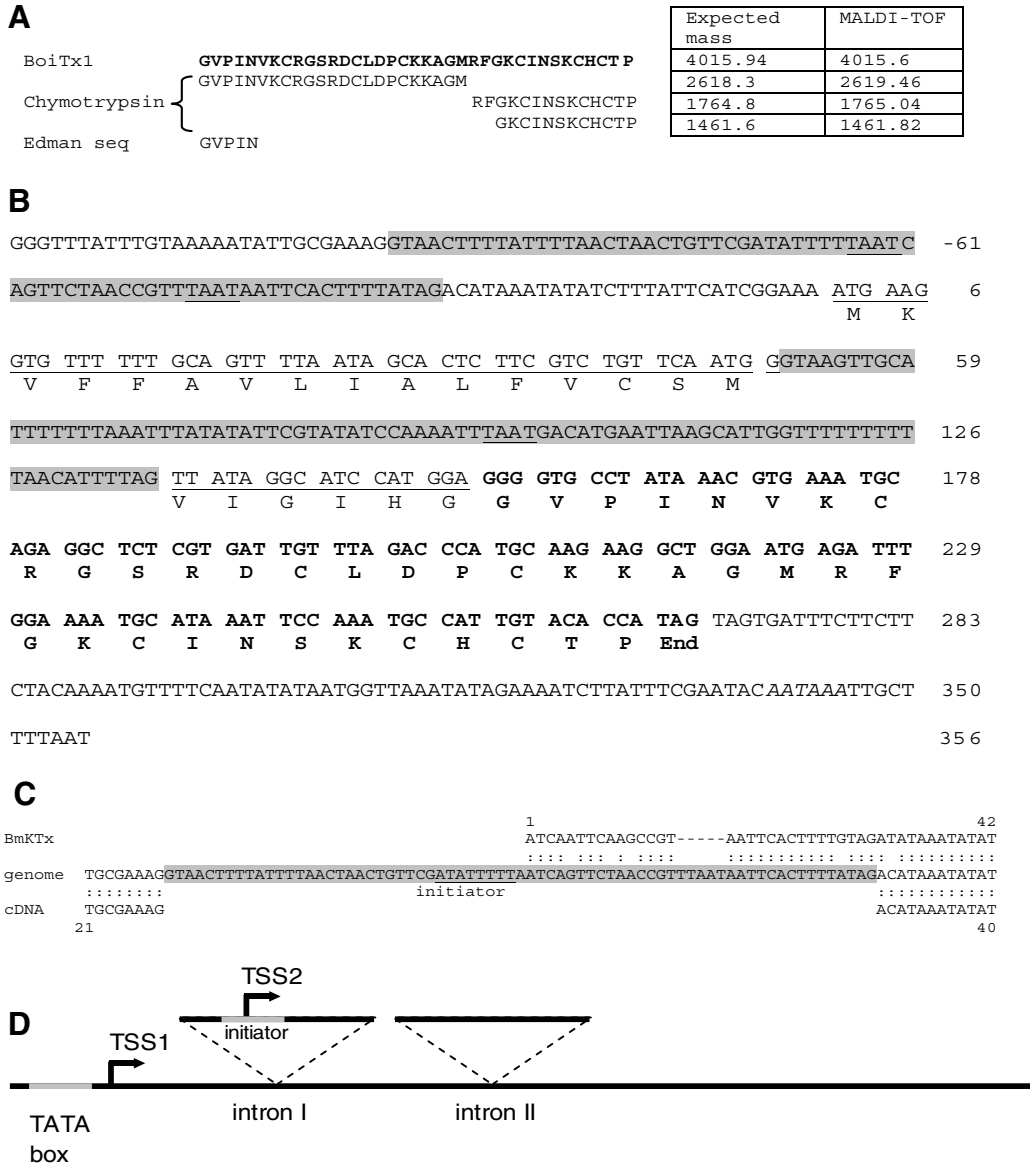

Fig. 2. Identification and genomic organization of BoiTx1. (A) Determination of the BoiTx1 sequence using MALDI-TOF analysis of the non-reduced toxin and its overlapping chymotrypsin-digested fragments. Masses given are those of the native mature peptide with its three disulfide bridges (−6 Da) and of the alkylated digested peptides (+171 Da per cysteine residue). Peptide monoisotopic masses are indicated. The results of Edman N-terminal sequence analysis are indicated at the bottom of the panel. (B) Nucleotide and deduced amino acid sequences of a BoiTx1 genomic clone. Nucleotides are numbered according to the first nucleotide coding the peptide. Intron sequences are highlighted in gray and putative branch points, 5′-TAAT-3′ [18,36], are underlined. The deduced amino acid sequence is given below the nucleotide sequence. The signal peptide is underlined. The mature toxin sequence is given in bold letters. The putative polyadenylation signal is marked in italics. (C) DNA sequence alignment of the BoiTx1 genomic gene to BoiTx1 and BmKTx cDNAs. A putative initiator sequence in underlined. Numbers correspond to the full-length cDNAs. Intron sequences are highlighted in gray. (D) Proposed structure of an α-KTx3 toxin gene. TATA box and initiator sites are shown in gray. Transcription start sites (TSS) are indicated by arrows.

deduced masses were compared with the database of expected masses, yielding only one match. In addition, the sequences of the first five amino acids of BoiTx1 were determined by Edman degradation (Fig. 2A). We thus conclude the amino acid sequence of BoiTx1 to be **GVPINVKCRGSRDCLDPCKKAGMRFGKCINSKCHCTP**.

3.3. Gene structure

The genomic organization of the BoiTx1 gene was found to be atypical of scorpion toxins genes. We identified two introns within the BoiTx1 gene (Fig. 2B). The first, 71 nucleotides long, is located within the 5′-untranslated region. The second, 88 nucleotide-long phase I intron, is located within the sequence coding for the leader sequence, typical of scorpion toxin genes [18]. The junctions of both introns are similar to those observed in various scorpion toxin genes, i.e., 5′-GTAAG/C and AG-3′ [18]. When aligning the sequence of cDNA clones of BoiTx1 and BmKTx (a member of the α-KTx3 family), no homology was detected in the first 33 nucleotides. Interestingly, this region showed 73.5% identity to the 3′ end of the first BoiTx1 intron (Fig. 2C), comparable to the overall 78.2% identity of the two cDNAs. In addition, a possible ini-

tiator element, similar to the initiator consensus sequence found in Mesotoxin [19,20], was detected within the first intron sequence. These findings enable us to offer a model for the gene organization and transcription of α-KTx3 toxins (Fig. 2D). We propose that all α-KTx3 genes contain two introns, like BoiTx1. In some genes, like that coding BoiTx1, transcription is initiated from a putative TATA box located upstream of the first intron. In other genes, like that coding BmKTx, transcription is initiated from an initiator located within the first intron. This possible alternative transcription mechanism would yield 5′-UTRs that vary in length, sequence and possibly mRNA stability. As two introns were also detected in other scorpion toxin genes [19,21,22], the proposed gene structure may not be unique to α-KTx3 toxins. It remains unclear whether the same gene can employ both transcription mechanisms.

3.4. Comparative sequence analysis

The cDNA product is translated into a 59 amino acid-long peptide, comprising a 22 amino acid-long leader peptide and a 37 amino acid-long mature toxin (Fig. 2B). The amino acid sequence of BoiTx1 was aligned using BLAST [23] and shared considerable identity of 63–84% with the members of the kaliotoxin family (α-KTx3; Fig. 5). We thus classified BoiTx1 as an α-KTx3.10 [5]. Members of this toxin family are known as blockers of voltage-gated potassium channels of the Kv1.X family [12,14]. Some kaliotoxins were also shown to block calcium-activated potassium channels (K_{Ca}) [13].

3.5. Electrophysiological studies

The biological effects of BoiTx1 were investigated in *X. laevis* oocytes heterologously expressing various potassium channels.

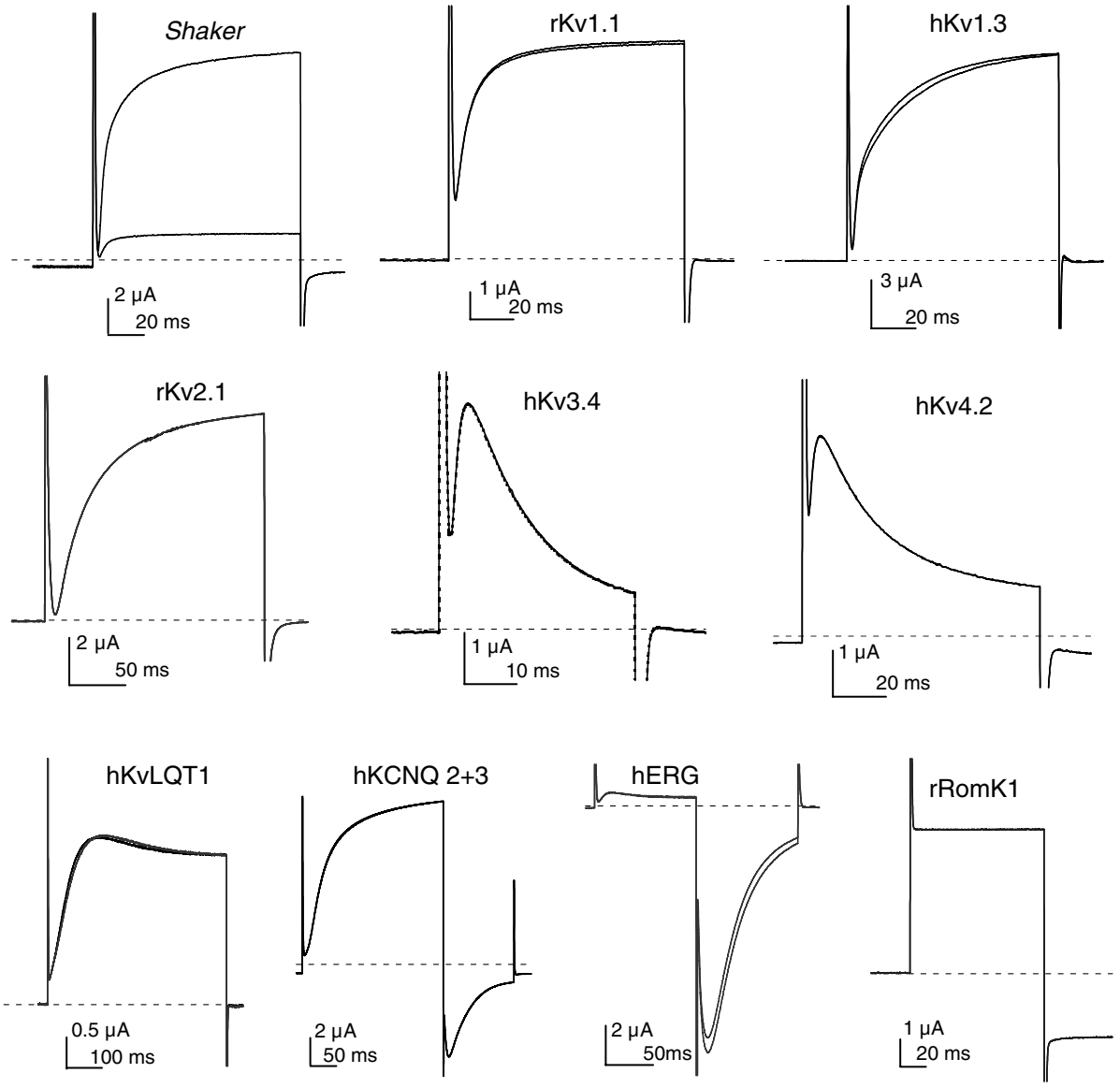

Fig. 3. Selected current traces showing the effect of 55 nM BoiTx1 on various potassium channels, as indicated. Channels were activated by depolarization from a holding potential of −60 mV to +40 mV, and then clamped back to −80 mV (*Shaker*, rKv1.1, hKv1.3, rKv2.1 and hKvLQT1) or by depolarization from a holding potential of −80 mV to +40 mV, and then clamped back to −120 mV (hKv3.4, hKv4.2, hKCNQ2 + 3, hERG and rRomK1). Currents before and after 1 min-long incubations with the toxin are indicated. Dotted lines indicate zero current. Only *Shaker* currents were considerably reduced.

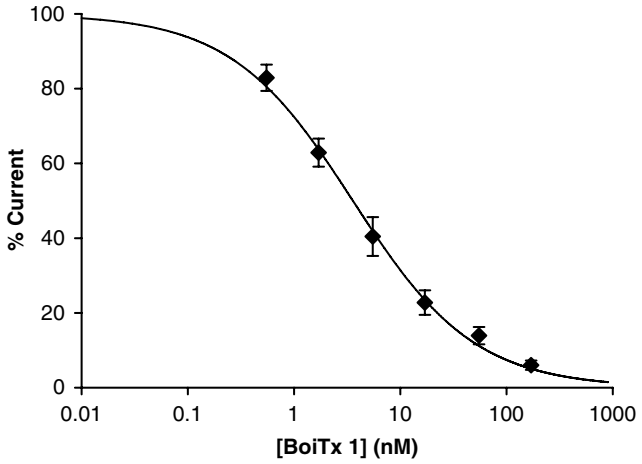

Fig. 4. Concentration-dependence of BoiTx1 activity towards *Shaker* channels. An IC_{50} value of 3.5 ± 0.5 nM was measured. Each point shown is the means ± S.E.M. from five experiments.

BoiTx1 (55 nM) reduced K^+ currents through *Drosophila Shaker* channels by 83 ± 2%, yet only had a minor effect on the structurally-related mammalian channels, rat Kv1.1 and human Kv1.3 (Fig. 3). Representatives of other mammalian potassium channels families (rat Kv2.1, rat RomK1 and human Kv3.4, Kv4.2, KvLQT1, KCNQ2 + 3 and ERG) were even less affected at the same BoiTx1 concentration (Fig. 3). Finally, while the IC_{50} value for *Shaker* channels was 3.5 ± 0.5 nM (Fig. 4), application of 550 nM BoiTx1 to either Kv1.1 or Kv1.3 resulted in less than 50% current reduction.

4. Discussion

In this study, we isolated, for the first time, a toxin from the venom of the Israeli scorpion *B. occitanus israelis*, termed BoiTx1, and found to be homologus to members of the α-KTx3 toxin family. As expected, and like its homologs, BoiTx1 was active against members of the Kv1.*X* potassium channel family (i.e. *Shaker*, Kv1.1 and Kv1.3). However, unlike other members of its family, BoiTx1 was at least 100-times more active against the fly *Shaker* channel than versus its mammalian counterparts. Accordingly, when injected into *Drosophila* larvae, BoiTx1 induced sudden and sustained contraction (not shown).

Towards elucidating the molecular basis of its action, a 3D model of BoiTx1 was generated (Fig. 6), according to the solved structures of other family members. The three critical residues for toxin-mediated Kv1.*X* potassium channel family blockade, Arg24, Lys27 and Asn30 [24], are present in BoiTx1 and located at similar positions as in related toxins. In addition, five of the six important residues for binding of Agitoxin2 to *Shaker* channels are present in BoiTx1 (i.e. Gly10, Ser11, Phe25, Thr36 and Pro37) [24]. The unique selectivity of BoiTx1 towards *Shaker* channels might be explained by structural differences that the toxin presents as compared to other members of its family. Firstly, BoiTx1 is one amino acid shorter than related toxins, lacking the C-terminal lysine residue normally found in most other toxin family members. This residue is predicted to be proximal to *Shaker* Glu422 [25]. Interestingly, this position is occupied by proline and alanine in the mammalian homologs Kv1.3 and Kv1.1 channels, respectively. In addition, the absence of the C-terminal Lys in BoiTx1 might affect the spatial position of the adjacent critical residue, Lys24. Secondly, Met29 was shown to participate in Agitoxin2 binding

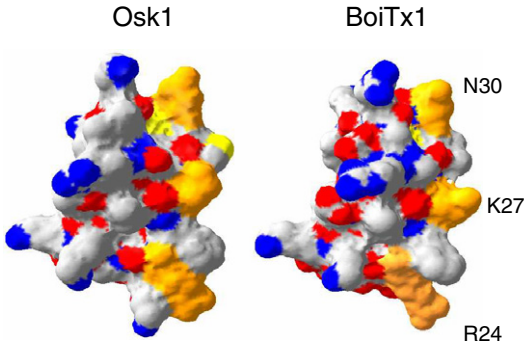

Fig. 6. Structural comparison of Osk1 and BoiTx1. A homology model of BoiTx1 is compared to the three-dimensional structure of Osk1 (PDB ID: 1SCO) [37]. The three critical residues for toxin-mediated Kv1.*X* potassium channel family blockade, Arg24, Lys27 and Asn30 [24], are highlighted in orange. The model structure was generated with SWISS-MODEL Protein Modeling Server (http://swissmodel.expasy.org) [41], based on the 3D structures of Osk1, AgTx2 and KTx1 (PDB ID: 1SCO, 1AGT and 2KTX, respectively).

Name	α-KTx	Sequence	%I
BoiTx1	3.10	*MKVFFAVLIALFVCSMVIGIHG***GVPINVKCRGSRDCLDPCKKAGMRFGKCINSKCHCTP-**	
AgTX1	3.4	------------------------T--PQ--K---D-------G-------K	84
OsK1	3.7	---------------------I----KI--Q--E-------M-G-------K	82
Bs6	3.8	------------------------PQ-IQ--RD-------M-G-------Q	79
KTX1	3.1	---------------------E----S--PQ--K---D-------M-R-------K	79
AgTX2	3.2	------------------------S-T--PQ-IK---D-------M-R-------K	76
AgTX3	3.3	------------------------P-T--PQ-IK---D-------M-R-------K	76
BmKTX	3.6	------T--I---I-----G-----KH-GQ--K---D---------G---D---K	74
KTX2	3.5	---S---I-------I---NA-R-P-S-KH-GQ--K---D-------M-G---D---K	66
KTX3	3.9	----------------------G-P-S-KH-GQ-IK---D-------M-R---D---K	63

Fig. 5. Amino acid sequence comparison of BoiTx1 with other toxins from the α-KTx3 family. The sequence of BoiTx1 was compared with that of AgTx1-3 from the venom of *Leiurus quinquestriatus hebraeus* [12], Osk1 from the venom of *Orthochirus scrobiculosus* [37], Bs6 from the venom of *Buthus sindicus* [38], BmKTx from the venom of *Buthus martensi* [39], KTx1 from the venom of *Androctonus mauretanicus mauretanicus* [40], KTx2 from the venom of *Androctonus australis* [13] and KTx3 from the venom of *Buthus occitanus tunetanus* [15]. Only amino acids that differ from those found in BoiTx1 at the same position are presented. Hyphens represent absent amino acids. Leader peptide amino acids are shown in italics. %I is the degree of identity a toxin shares with BoiTx1. Note that only the leader peptide amino acid sequences of α-KTx3.5, 3.6 and 3.9 are known.

to *Shaker* channels [24]. By contrast, Ile29 is found in BoiTx1 and Agitoxin1, both of which display reduced activity towards Kv1.3 channels, as compared to Agitoxin2, but high potency towards *Shaker* channels [12]. Finally, we cannot rule out the possibility that the unique selectivity of BoiTx1 is due to the presence of residues adjacent to the binding site that selectively destabilize binding to Kv1.3 channels.

Potent peptide toxins and their derivatives constitute good lead compounds for the development of novel insecticides [26–28]. Scorpion toxins cannot penetrate through insect gut in their native state. Accordingly, various solutions have been offered to increase their permeability into the circulatory system of target animals, including fusion to the snowdrop lectin (*Galanthus nivalis* agglutinin) [29], expression in plant cells in parallel to chitinase for digestion of the insect gut membrane [30], or the use of insect-selective baculoviruses engineered with genes encoding insect-selective toxins [31–35]. To date, such approaches have only been employed with scorpion toxins affecting insect sodium channels. Here, we suggest a new target for insect-selective insecticides, i.e., the potassium voltage-gated channel. The enhancement of the neuronal sodium current mediated by scorpion venom is accompanied by a blockage of the potassium voltage-gated current, so as to maximize the toxic effect. The concurrent use of an anti-insect-specific potassium channel blocker would thus be expected to augment the insecticidal effects of currently-used sodium channel modifiers. Therefore, we propose BoiTx1 as a lead compound for engineering a novel class of potential insecticides.

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References

[1] Carbone, E., Wanke, E., Prestipino, G., Possani, L.D. and Maelicke, A. (1982) Selective blockage of voltage-dependent K^+ channels by a novel scorpion toxin. Nature 296, 90–91.
[2] Possani, L.D., Martin, B.M. and Svendsen, I. (1982) The primary structure of noxiustoxin: a K^+ channel blocking peptide, purified from the venom of the scorpion *Centruroides noxius Hoffmann*. Carlsberg Res. Commun. 47, 285–289.
[3] Garcia, M.L., Hanner, M. and Kaczorowski, G.J. (1998) Scorpion toxins: tools for studying K^+ channels. Toxicon 36, 1641–1650.
[4] Garcia, M.L., Hanner, M., Knaus, H.G., Slaughter, R. and Kaczorowski, G.J. (1999) Scorpion toxins as tools for studying potassium channels. Methods Enzymol. 294, 624–639.
[5] Rodriguez de la Vega, R.C. and Possani, L.D. (2004) Current views on scorpion toxins specific for K^+-channels. Toxicon 43, 865–875.
[6] Tytgat, J., Chandy, K.G., Garcia, M.L., Gutman, G.A., Martin-Eauclaire, M.F., van der Walt, J.J. and Possani, L.D. (1999) A unified nomenclature for short-chain peptides isolated from scorpion venoms: alpha-KTx molecular subfamilies. Trends Pharmacol. Sci. 20, 444–447.
[7] Abdel-Mottaleb, Y., Coronas, F.V., de Roodt, A.R., Possani, L.D. and Tytgat, J. (2006) A novel toxin from the venom of the scorpion *Tityus trivittatus*, is the first member of a new alpha-KTX subfamily. FEBS Lett. 580, 592–596.
[8] Rodriguez de la Vega, R.C., Merino, E., Becerril, B. and Possani, L.D. (2003) Novel interactions between K^+ channels and scorpion toxins. Trends Pharmacol. Sci. 24, 222–227.
[9] Gurevitz, M. and Zilberberg, N. (1994) Advances in molecular genetics of scorpion neurotoxins. J. Toxicol. – Toxin Rev. 13, 65–100.
[10] Zlotkin, E., Miranda, F. and Lissitzky, S. (1972) Proteins in scorpion venoms toxic to mammals and insects. Toxicon 10, 207–209.
[11] Zlotkin, E., Rochat, H., Kopeyan, C., Miranda, F. and Lissitzky, S. (1971) Purification and properties of the insect toxin from the venom of the scorpion *Androctonus australis Hector*. Biochimie 53, 1073–1078.
[12] Garcia, M.L., Garcia-Calvo, M., Hidalgo, P., Lee, A. and MacKinnon, R. (1994) Purification and characterization of three inhibitors of voltage-dependent K^+ channels from *Leiurus quinquestriatus* var. *hebraeus* venom. Biochemistry 33, 6834–6839.
[13] Laraba-Djebari, F., Legros, C., Crest, M., Ceard, B., Romi, R., Mansuelle, P., Jacquet, G., van Rietschoten, J., Gola, M., Rochat, H., Bougis, P.E. and Martin-Eauclaire, M.F. (1994) The kaliotoxin family enlarged. Purification, characterization, and precursor nucleotide sequence of KTX2 from *Androctonus australis* venom. J. Biol. Chem. 269, 32835–32843.
[14] Mouhat, S., Visan, V., Ananthakrishnan, S., Wulff, H., Andreotti, N., Grissmer, S., Darbon, H., De Waard, M. and Sabatier, J.M. (2005) K^+ channel types targeted by synthetic OSK1, a toxin from *Orthochirus scrobiculosus* scorpion venom. Biochem. J. 385, 95–104.
[15] Meki, A., Mansuelle, P., Laraba-Djebari, F., Oughideni, R., Rochat, H. and Martin-Eauclaire, M.F. (2000) KTX3, the kaliotoxin from *Buthus occitanus tunetanus* scorpion venom: one of an extensive family of peptidyl ligands of potassium channels. Toxicon 38, 105–111.
[16] Levi, G. and Amitai, P. (1980) Fauna Palaestina, Arachnida I: Scorpiones, The Israel Academy of Sciences and Humanities.
[17] Zlotkin, E. and Gordon, D. (1985) Detection, purification and receptor binding assays of insect selective neurotoxins derived from scorpion venoms in: Neurochemical Techniques in Insect Research (Miller, T.A. and Breer, H., Eds.), pp. 243–295, Springer-Verlag, Berlin.
[18] Froy, O., Sagiv, T., Poreh, M., Urbach, D., Zilberberg, N. and Gurevitz, M. (1999) Dynamic diversification from a putative common ancestor of scorpion toxins affecting sodium, potassium, and chloride channels. J. Mol. Evol. 48, 187–196.
[19] Zhu, S. and Gao, B. (2006) Molecular characterization of a possible progenitor sodium channel toxin from the Old World scorpion *Mesobuthus martensii*. FEBS Lett. 580, 5979–5987.
[20] Lodish, H., Berk, A., Zipursky, S.L., Matsudaira, P., Baltimore, D. and Darnell, J. (2000) Molecular Cell Biology, W.H. Freeman and Company, New York.
[21] Ji, Y.H., Wang, W.X., Ye, J.G., He, L.L., Li, Y.J., Yan, Y.P. and Zhou, Z. (2003) Martentoxin, a novel K^+-channel-blocking peptide: purification, cDNA and genomic cloning, and electrophysiological and pharmacological characterization. J. Neurochem. 84, 325–335.
[22] Zhijian, C., Yun, X., Chao, D., Shunyi, Z., Shijin, Y., Yingliang, W. and Wenxin, L. (2006) Cloning and characterization of a novel calcium channel toxin-like gene BmCa1 from Chinese scorpion *Mesobuthus martensii Karsch*. Peptides 27, 1235–1240.
[23] Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Zhang, Z., Miller, W. and Lipman, D.J. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25, 3389–3402.
[24] Ranganathan, R., Lewis, J.H. and MacKinnon, R. (1996) Spatial localization of the K^+ channel selectivity filter by mutant cycle-based structure analysis. Neuron 16, 131–139.
[25] Eriksson, M.A. and Roux, B. (2002) Modeling the structure of agitoxin in complex with the Shaker K^+ channel: a computational approach based on experimental distance restraints extracted from thermodynamic mutant cycles. Biophys. J. 83, 2595–2609.
[26] Nicholson, G.M. (2007) Fighting the global pest problem: preface to the special Toxicon issue on insecticidal toxins and their potential for insect pest control. Toxicon 49, 413–422.
[27] Gurevitz, M., Karbat, I., Cohen, L., Ilan, N., Kahn, R., Turkov, M., Stankiewicz, M., Stuhmer, W., Dong, K. and Gordon, D. (2007) The insecticidal potential of scorpion beta-toxins. Toxicon 49, 473–489.

[28] Gordon, D., Karbat, I., Ilan, N., Cohen, L., Kahn, R., Gilles, N., Dong, K., Stuhmer, W., Tytgat, J. and Gurevitz, M. (2007) The differential preference of scorpion alpha-toxins for insect or mammalian sodium channels: Implications for improved insect control. Toxicon 49, 452–472.
[29] Pham Trung, N., Fitches, E. and Gatehouse, J.A. (2006) A fusion protein containing a lepidopteran-specific toxin from the South Indian red scorpion (*Mesobuthus tamulus*) and snowdrop lectin shows oral toxicity to target insects. BMC Biotechnol. 6, 18.
[30] Wang, J., Chen, Z., Du, J., Sun, Y. and Liang, A. (2005) Novel insect resistance in *Brassica napus* developed by transformation of chitinase and scorpion toxin genes. Plant Cell Rep. 24, 549–555.
[31] Chejanovsky, N., Zilberberg, N., Rivkin, H., Zlotkin, E. and Gurevitz, M. (1995) Functional expression of an alpha anti-insect scorpion neurotoxin in insect cells and lepidopterous larvae. FEBS Lett. 376, 181–184.
[32] McCutchen, B.F., Choudary, P.V., Crenshaw, R., Maddox, D., Kamita, S.G., Palekar, N., Volrath, S., Fowler, E., Hammock, B.D. and Maeda, S. (1991) Development of a recombinant baculovirus expressing an insect-selective neurotoxin: potential for pest control. Biotechnology (NY) 9, 848–852.
[33] Stewart, L.M., Hirst, M., Lopez Ferber, M., Merryweather, A.T., Cayley, P.J. and Possee, R.D. (1991) Construction of an improved baculovirus insecticide containing an insect-specific toxin gene. Nature 352, 85–88.
[34] Regev, A., Rivkin, H., Inceoglu, B., Gershburg, E., Hammock, B.D., Gurevitz, M. and Chejanovsky, N. (2003) Further enhancement of baculovirus insecticidal efficacy with scorpion toxins that interact cooperatively. FEBS Lett. 537, 106–110.
[35] Whetstone, P.A. and Hammock, B.D. (2007) Delivery methods for peptide and protein toxins in insect control. Toxicon 49, 576–596.
[36] Senapathy, P., Shapiro, M.B. and Harris, N.L. (1990) Splice junctions, branch point sites, and exons: sequence statistics, identification, and applications to genome project. Methods Enzymol. 183, 252–278.
[37] Jaravine, V.A., Nolde, D.E., Reibarkh, M.J., Korolkova, Y.V., Kozlov, S.A., Pluzhnikov, K.A., Grishin, E.V. and Arseniev, A.S. (1997) Three-dimensional structure of toxin OSK1 from *Orthochirus scrobiculosus* scorpion venom. Biochemistry 36, 1223–1232.
[38] Ali, S.A., Stoeva, S., Schutz, J., Kayed, R., Abassi, A., Zaidi, Z.H. and Voelter, W. (1998) Purification and primary structure of low molecular mass peptides from scorpion (*Buthus sindicus*) venom. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 121, 323–332.
[39] Romi-Lebrun, R., Lebrun, B., Martin-Eauclaire, M.F., Ishiguro, M., Escoubas, P., Wu, F.Q., Hisada, M., Pongs, O. and Nakajima, T. (1997) Purification, characterization, and synthesis of three novel toxins from the Chinese scorpion *Buthus martensi*, which act on K^+ channels. Biochemistry 36, 13473–13482.
[40] Crest, M., Jacquet, G., Gola, M., Zerrouk, H., Benslimane, A., Rochat, H., Mansuelle, P. and Martin-Eauclaire, M.F. (1992) Kaliotoxin, a novel peptidyl inhibitor of neuronal BK-type Ca^{2+}-activated K^+ channels characterized from *Androctonus mauretanicus mauretanicus* venom. J. Biol. Chem. 267, 1640–1647.
[41] Schwede, T., Kopp, J., Guex, N. and Peitsch, M.C. (2003) SWISS-MODEL: an automated protein homology-modeling server. Nucleic Acids Res. 31, 3381–3385.